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Effect of bioagent formulations on progress of bacterial leaf blight disease of rice under field conditions

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Abstract: In the present study, effectiveness of different fungal (*Trichoderma harzianum*) and bacterial (*Pseudomonas fluorescens*) bioagent formulations in reducing progress of the bacterial leaf blight disease of rice under field conditions was studied and compared with chemical treatment and untreated check. The results exhibited that after 23 to 30 days after first application, bioagent formulations were more effective then chemical treatment in reducing progress of disease. Bioagent formulations exhibited long lasting effect in reducing progress of disease during *Kharif*, 2006 and 2007. Application of bioagent formulations resulted in significant reduction (60.5 – 142.8%) in area under disease progress curve (AUDPC) as compared to check during *Kharif*, 2006 and 2007. Significant increase in grain yield (14.3 - 21.5 %) was observed with the application of bioagent formulations as compared to check during *Kharif*, 2006 and 2007.

Keywords: AUDPC, Bacterial leaf blight of rice, Disease progress curve, Infection rate, *Pseudomonas fluorescens*, *Trichoderma harzianum*

INTRODUCTION

Bioagents offers several advantages over chemical control like they are more stable without development of resistance in pathogen. Bioagents are non-phytotoxic and causes little disturbance in ecological balance. These are safe to environment, animal and human health and may also influence the ecological factors in the favour of crop or mitigating the effect of pathogen (Singh *et al.*, 2005). Bioagents are reported to stimulate plant growth, even if there is no disease which results in better yield (Mishra and Sinha, 2000). Various antagonists (*Trichoderma* spp. *T. harzianum, Bacillus* spp., *Pseudomonas* spp., *P. putida, P. fluorescence, Erwinia Herbicola* and phylloplane microflora) are known to exhibits inhibitory effect on bacterial leaf blight disease of rice (Manmeet and Thind, 2002; Nzojiyobiri *et al.*, 2003 and Rangarajan *et al.*, 2003; Gangwar and Sinha, 2012a,b,c; Gangwar, 2012 and Gangwar, 2013). Bacterial leaf blight of rice has caused enormous losses in India and in all the rice growing areas of the world (Mew *et al.*, 1993 and Anonymous, 2002). Outbreak of the disease is favoured by combination of metrological factors such as high temperature, high humidity, heavy rain fall, high light intensity and frequent typhoons (Murlidharan and Venkatarao, 1979). Mew *et al.*, (1993) studied progress of disease related to plant growth stages like, seedbed, seedlings, panicle initiation, flowering and mature grain stages. Disease progress curves, apparent infection rate and AUDPC for bacterial leaf blight of rice have been developed by several workers (Adhikari *et al.*, 1994; Ahmed *et al.*, 1997 and Oña *et al.*, 1998). Pattern of Disease progress curves (DPCs), apparent infection rate (AIR) and area under disease progress curve (AUDPC) provide better understanding of progress of disease. Analyzing these epidemiological parameters helps in predicting the epidemic on set, reaction of host plant, expected disease severity and yield loss. Effectiveness of bioagents against different plant diseases was reported by several workers by the assessment of infection rate and AUDPC (Elmer and McGovern, 2004; Verma and Dohroo, 2005 and Daghman *et al.*, 2006). In the present investigation, effect of bioagent formulations and chemical treatment on the progress of bacterial leaf blight disease of rice was observed using different epidemiological parameters *viz.* disease progress curve (DPC), infection rate and area under disease progress curve (AUDPC).

MATERIALS AND METHODS

This experiment was conducted in *Kharif* season during the years 2006 and 2007 at Crop Research Centre, G. B. Pant University of Agriculture and Technology, Pantnagar. Susceptible rice cultivar Jaya was used for the experiment. General agronomic practices were followed for the cultivation of experimental plots. Influence of two *P. fluorescens* formulations (*Pf* 83 and PBA-2), two *T. harzianum* formulations (*T. harzianum* and PBA-1) and one mixed formulation of *P. fluorescens* + *T. harzianum*

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formulation (PBA-3) on the progress of bacterial leaf blight of rice were studied along with chemical treatment [streptocycline (0.03 g/ litre water) + copper oxychloride (1 g/litre water)] and untreated check. Bioagent formulations (10^6 cfu/g) were applied @10 g / litre water. All treatments were applied twice at 7 days interval in experimental plots using randomized block design.

Preparation of bioagent formulations: *T. harzianum* was mass multiplied on barnyard millet (*Echinocloa frumentacae*). Grains colonized by *Trichoderma* were air dried in open shade and ground with the help of Willy Mill to get fine powder. This powder was passed through 50 and 80 mesh sieves simultaneously to obtain spore powder. However *P. fluorescens* was mass multiplied on King's B broth. Both spore powder and broth culture diluted with talcum powder (mesh = 350 with 95% whiteness) and 1% carboxyl methyl cellulose (CMC) to get desired concentration (10^6 cfu/g) of bioagents in the formulation.

Inoculation of pathogen and treatment application: Pathogen was inoculated by clipping off the leaf tip @ 10^6 cell/ml inoculum (Kauffman *et al.* 1973). Bioagent formulations and chemical treatment were applied next day of pathogen inoculation. Successive application of treatments was given after 7 days interval of first application. Data on percent disease severity recorded 14 days after first spray at 3 days interval. Disease progress curves were developed by plotting disease severity (%) against time. Grain yield was recorded after harvesting.

Calculation for infection rate: Apparent infection rate was recorded for 3 days interval by using following formula (Vanderplank, 1963):

$$r = \frac{2.3}{t_2 - t_1} \log 10 \frac{x_2 (1 - x_1)}{x_1 (1 - x_2)}$$

Where,

x_1= Disease index at time t_1(time of first disease rating)

x_2= Disease index at time t_2 (time of second disease rating)

r = Apparent infection rate

Disease severity (%) was converted in to unit, by dividing with 100 and used in place of disease index for calculating apparent infection rate of bacterial leaf blight of rice.

Calculation for area under disease progress curve (AUDPC): Area under disease progress curve (AUDPC) was calculated by using following formula (Shanner and Finney, 1977):

$$AUDPC = \frac{\left(\frac{D_1 + D_2}{2} \times T\right) + \left(\frac{D_2 + D_3}{2} \times T\right) \ldots\ldots \left(\frac{D_{n-1} + D_n}{2} \times T\right)}{n - 1}$$

Where, D = Percent disease severity at different dates ($D_1, D_2, D_3 \ldots\ldots D_n$)

T = Time interval (days) between two observations

n = Total number of observations

RESULTS AND DISCUSSION

Effect on disease progress curves: Disease progress curves were drawn for disease developing in experimental plots with different treatments. It was observed that DPCs for bioagent formulations revealed clear cut and equal length of lag, log and decline phase (Fig. 1). However, chemical treatment showed prolong lag and log phase. Decline phase was not observed with chemical treatment. In check, short lag and prolonged log phase were observed during *Kharif* season of 2006. However, during Kharif 2007 DPCs for bioagent formulations showed prolong lag and log phase which were not distinct (Fig. 2) and decline phase were not observed. Chemical treatment showed prolong lag and log phase. Check plots showed short lag phase followed by sharp increased in log phase with no decline phase.

A disease progress curve, AUDPC and the epidemic rate were calculated by Monaco *et al.* (1999) with Saprobic fungi (*Nigrospora* spp., *Penicillium* sp. b, *Chaetomium globosum*, *Cladosporium cladosporioides* and *Trichoderma polysporum*) which inhabited *Alternaria solani* in tomato phylloplane. In the present study, disease progress curves depicting progress of bacterial leaf blight of rice, under field condition were developed for bioagent formulations and compared with chemical treatments and untreated check (Fig. 1, 2). DPCs of bioagent formulations were showed short leg and log phase which was followed by prolong decline phase. Short leg and log phase may be because bioagent formulations did showed effectivity sooner as they applied and later they showed higher effectivity in reducing progress of disease and hence resulted prolong decline phase. Delayed and prolong leg phase was showed by chemical treatment showing instant effectivity of chemical treatment which was not long lasting and hence showed sharp log phase due to higher progress of disease. Decline phase was absent as total mortality of leaves occurred. DPC for untreated check, showed very short leg phase which was followed by long log phase showing fastest progress of disease. After killing all tissues sharp decline phase was observed as there is no fresh tissues available for further infection. However, DPC with chemical treatments was lagging behind the DPCs with bioagent formulations showing higher effectivity of chemicals over bioagent formulations at initial stage of disease progress. Later DPC of chemical treatment crossed over the DPCs of bioagents and crossing above. This may be explained as during later stage of disease progress bioagents have got sufficient time for population build up and/or induces resistance and hence showed higher effectivity over chemical treatment in due course of time. The effectivity of bioagent formulations was long lasting while, chemical showed effectivity in

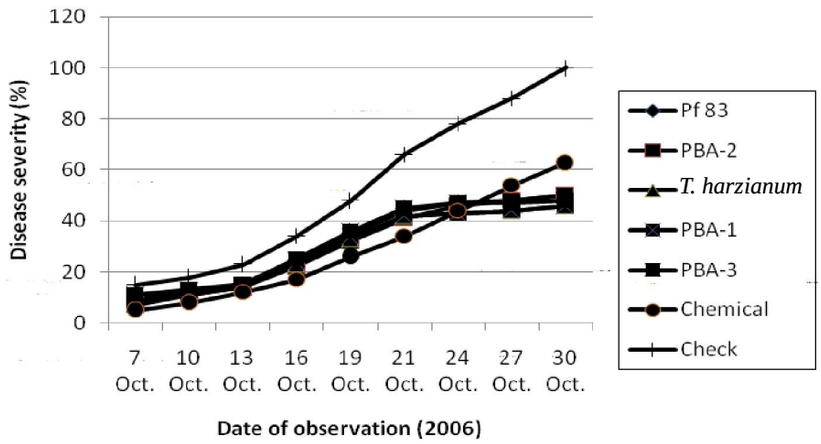

Fig. 1. *Effect of application of bioagent formulations on progress of bacterial leaf blight disease severity, during Kharif 2006. *Mean of three replications; P. fluorescens formulations (Pf 83 and PBA-2), T. harzianum formulations (T. harzianum and PBA-1) and T. harzianum + P. fluorescens formulation (PBA-3).*

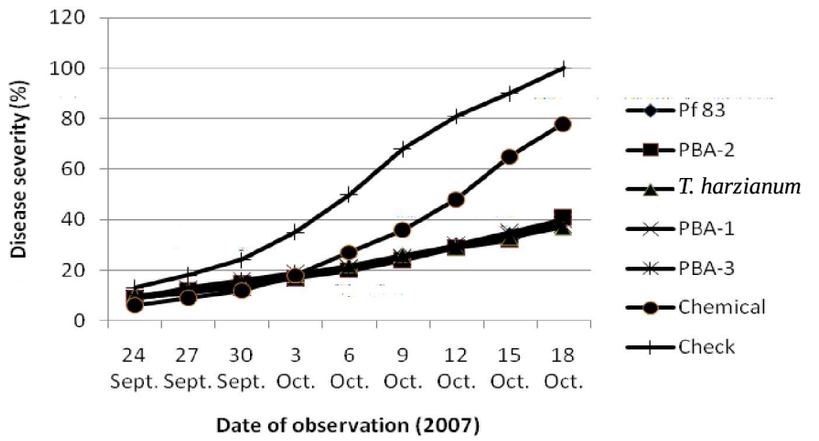

Fig. 2. *Effect of application of bioagent formulations on progress of bacterial leaf blight disease severity, during Kharif 2007. *Mean of three replications; P. fluorescens formulations (Pf 83 and PBA-2), T. harzianum formulations (T. harzianum and PBA-1) and T. harzianum + P. fluorescens formulation (PBA-3).*

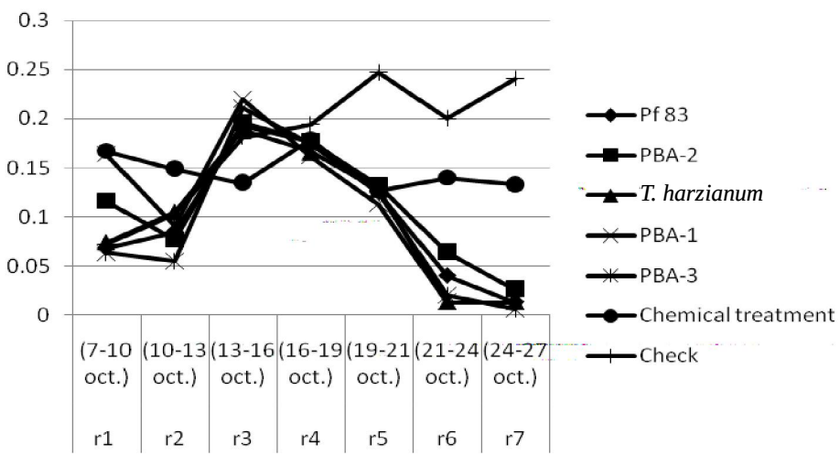

Fig. 3. *Effect of application of bioagent formulations on infection rate of bacterial leaf blight disease at three days interval during Kharif 2006. *Mean of three replications; P. fluorescens formulations (Pf 83 and PBA-2), T. harzianum formulations (T. harzianum and PBA-1) and T. harzianum + P. fluorescens formulation (PBA-3)*

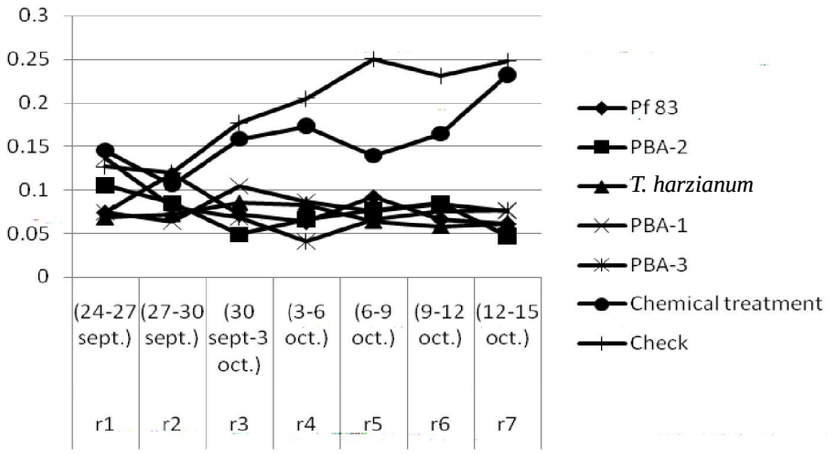

Fig. 4. *Effect of application of bioagent formulations on infection rate of bacterial leaf blight disease at three days interval during Kharif 2007. *Mean of three replications; P. fluorescens formulations (Pf 83 and PBA-2), T. harzianum formulations (T. harzianum and PBA-1) and T. harzianum + P. fluorescens formulation (PBA-3)*

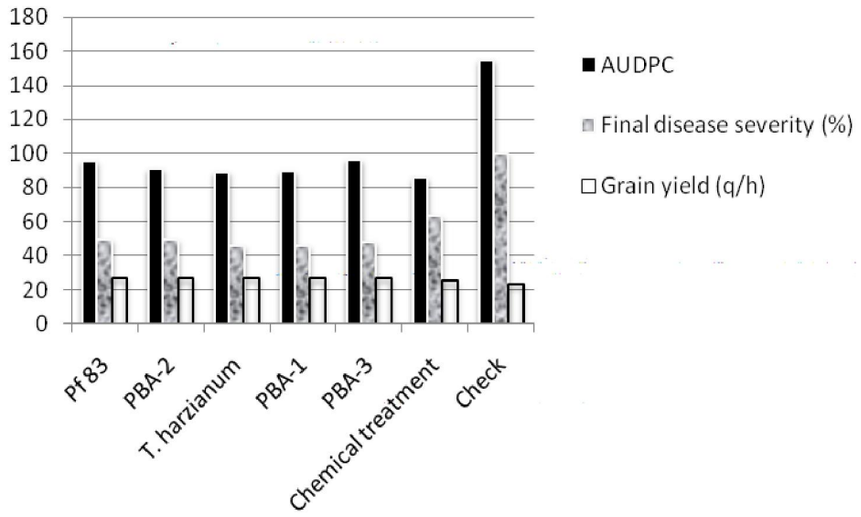

Fig. 5. *Effect of application of bioagent formulations on area under disease progress curve (AUDPC) for bacterial leaf blight disease and grain yield (q/h) of rice during Kharif 2006. *Mean of three replications; P. fluorescens formulations (Pf 83 and PBA-2), T. harzianum formulations (T. harzianum and PBA-1) and T. harzianum + P. fluorescens formulation (PBA-3).*

checking the progress of disease for a certain period of time.

Effect on infection rate: During cropping season *Kharif* 2006, infection rates were calculated for all treatments. At early stage of disease progress (r_1, r_2 and r_3) were found statistically similar to check plots. However, at later stage of disease progress significantly reduced infection rates (r_4, r_5, r_6 and r_7) with application bioagent formulations were observed as compared to check (Fig. 3). Reduced infection rates in late stages of disease progress (r_6 and $r_{7)}$ were observed for all bioagents as compared to chemical treatment and check. During cropping season *Kharif* 2007, at early stage of disease progress bioagent formulations PBA-3, *Pf* 83 and chemical treatment showed r_1 and r_2 statistically similar to check. As disease progressed, significantly lowered infection rates (r_3, r_4, r_5, r_6 and r_7) were observed with application of bioagent formulations as compared to check and chemical treatment (Fig. 4).

Effectiveness of bioagents against different plant diseases was reported by several workers (Elmer and McGovern, 2004; Verma and Dohroo, 2005 and Daghman *et al.*, 2006) by the assessment of infection rate and AUDPC. Minimum apparent infection rate, decreased AUDPC and increased seed germination was observed by Verma and Dohroo (2005) against *Fusarium* wilt of pea with the bioagents, *T. viride* and *T. harzianum* as compared to control. In the present study, infection rates during early stage of disease progress (r_1, r_2 and r_3 during *Kharif* 2006 and r_1 and r_2 during *Kharif* 2007) with bioagent formulations were equal to that of check plots (Tables 1, 2). This may be because bioagent formulations were not

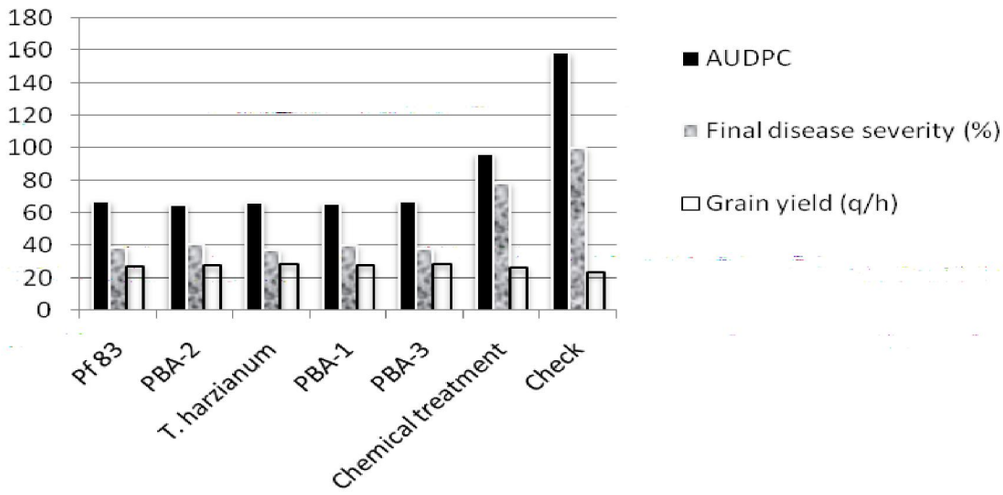

Fig. 6. *Effect of application of bioagent formulations on area under disease progress curve (AUDPC) for bacterial leaf blight disease and grain yield (q/h) of rice during Kharif 2007.*Mean of three replications; P. fluorescens formulations (Pf 83 and PBA-2), T. harzianum formulations (T. harzianum and PBA-1) and T. harzianum + P. fluorescens formulation (PBA-3).*

effective in reducing progress of disease in early stage. However, in later stage of disease progress infection rates (r_4, r_5, r_6 and r_7 during *Kharif* 2006 and r_3, r_4, r_5, r_6 and r_7 during *Kharif* 2007) with bioagent formulations were lowered as compared to check which reveals higher effectivity of bioagent formulations over check. Infection rates, r_6 and r_7 during *Kharif* 2006 and r_3, r_4, r_5, r_6 and r_7 during *Kharif* 2007 was found lower as compared to chemical treatment showing higher effectivity of bioagent formulations over chemical treatments in later stage of disease progress. Infection rate declined in case of bioagents it might be due to population build up of antagonists and/or induced resistance. Increasing infection rate over time observed in case of chemical is expected as their impact was quick and short lived and indicating decrease in the performance of chemicals over time.

Effect on area under disease progress curve (AUDPC): All treatments exhibited significantly lowered AUDPC as compared to check (Fig. 5) during *Kharif* 2006. Application of *T. harzianum* Formulation showed maximum reduction (42.34%) in AUDPC followed by PBA-1 (42.22%) and PBA-2 (41.20%). During *Kharif* 2007, all treatments were effective in reducing AUDPC as compared to check and chemical treatment (Fig. 6). Maximum reduction in AUDPC was recorded with Application of PBA-2 formulation (58.81%) which was followed by PBA-1 (58.22%) and *T. harzianum* (57.98%). Based on disease incidence expressed as the AUDPC, Daghman *et al.* (2006) concluded that the *T. harzianum* (UPM40) dry preparation was effective in protecting the seeds and seedlings against pre and post emergence damping-off caused by *Rhizoctonia solani* in leaf mustard (*Brassica rapa*). In the present study, during cropping season *Kharif* 2006, PBA-2, *Pf* 83 and PBA-3 formulations showed lower values of AUDPC as compared to chemical treatment and check (Fig. 5). Under field condition, bioagent formulations were more effective than chemical treatments in reducing amount of disease. However during cropping season *Kharif* 2007, all bioagent formulations showed significantly reduced AUDPC as compared to check and chemical treatment (Fig. 6). This revealed higher effectivity of bioagent formulations over chemical treatment under field condition. Elmer and McGovern (2004) reported reduction in the AUDPC when bioagents were applied following combinations of a fungicide treatment or tank-mixed with a fungicide which indicated the suppression of *Fusarium* wilt of cyclamen. Raupach *et al.* (1996) observed significantly lower AUDPC with *P. fluorescens* strain 89B-27 than in the nonbacterized control while working on induced systemic resistance in cucumber and tomato against cucumber mosaic cucumovirus using *P. fluorescens* strains 89B-27 and *Serratia marcescens* strain 90-166.

Conclusion

The present study concluded that after a period of time of 23 to 30 days after first application, all bioagent formulations were more effective then chemical treatment in reducing progress of disease. Bioagent formulations exhibited long lasting effect in reducing progress of disease in both the crop seasons. Their applications resulted in significant reduction in area under disease progress curve (AUDPC) as compared to check and chemical treatment. Further study is needed to understand the mechanism of reduction in epidemiological parameters due to application of these bioagent formulations.

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